bio-protocol Preprint

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Cell preparation for analysis

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An abbreviated version of this protocol was published in eLIFE in Mar 2020

Tumors attenuating the mitochondrial activity in T cells escape from PD-1 blockade therapy

DOI: 10.7554/eLife.52330

Detailed protocol

For GL-261, I used 2 million cells in 100ul PBS suspension. After harvesting the cells, washed two times with PBS and maintained the cell density at 20 million cells/mL and mice were intradermally injected on right flank with 100uL suspension containing 2 million cells. On the day of analysis, mice were sacrificed and tumors were harvested. Tumor weight was measured. Tumor was minced with scissor for one minute to make 1mm small pieces about. 3 mL of Collagenase type IV solution (1mg/mL conc. in PBS; Worthington Biochemical Corporation, Lakewood, NJ, Catalog # LS004188) was added and dissociated into single cell suspension using a gentle MACS Dissociator (Miltenyi Biotec). After the completion of digestion process (around 4o minutes) 7 ml complete RPMI (with FCS and P/S) media were added, centrifuged. Pellets were resuspended in 3 mL and filtered using 70u filter. Cells were counted. Cells can be stained with PI (for viability), anti-CD8 mAb, anti-CD45.2 mAb and other antibodies of your interest. The PI- CD8+ CD45.2+ cells are the TILs that infiltrated in the tumor mass.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Kumar, A. (2020). Cell preparation for analysis. Bio-protocol Preprint. bio-protocol.org/prep561.
2. Kumar, A., Chamoto, K., Chowdhury, P. S. and Honjo, T.(2020). Tumors attenuating the mitochondrial activity in T cells escape from PD-1 blockade therapy. eLIFE. DOI: 10.7554/eLife.52330